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Chapter

Kinetic Studies on Cell Growth

Punniavan Sakthiselvan, Setti Sudharsan Meenambiga and Ramasamy Madhumathi

Abstract

The kinetic model of cell growth is substantially capable to predict product formation. Mathematical models provide a strategy for solving problems encountered in fermentation process. A biochemical engineering approach to address this problem could be to develop a mathematical model which not only helps in the understanding of the system but also predicts various cultivation strategies to facilitate the optimization of a fermentation process, saving much of the time and cost for performing experiments. The presented overview indicates that many of the environmentally relevant aspects in growth kinetics are still waiting to be discovered, established, and exploited. A kinetic model that describes microbial growth, product formation and substrate consumption and the experimental data were fitted with modified logistic equation.

Keywords: cell growth, kinetics, fermentation, biomass, growth associated, product

1. Introduction

Cell growth implies increase in its mass and physical size controlled by physical, biological and chemical environments. Microbial growth is quantified by increase in the macromolecular and chemical constituents of the cell and growth pattern of each microbe is unique. Cell growth and cell division are inseparable for microbes as bacteria divide by binary fission, yeast cells by budding and viruses divide intracellularly [1]. Microbial growth during log phase is very important for the analysis of cells due to division by binary fission [2]. A typical mammalian cell growth is influenced by nutrient availability and thus a threshold cell size is required for DNA synthesis and mitosis [3]. Thus, each class of organisms have a different growth pattern based on their cell cycle and cell division. Understanding the growth kinetics of different classes of organisms forms the basis for fermentation process to achieve optimum product concentration.

Growth kinetics is an autocatalytic reaction which implies that the rate of growth is directly proportional to the concentration of cell. The cell concentration is measured by direct and indirect methods. Direct methods include measuring the cell mass concentration and cell number density by its dry weight, turbidity (optical density), plate counts etc. Whereas, indirect methods of measuring cell density are done by measuring the concentration of proteins, ATP or DNA content [4].

Batch growth kinetics of a microbe follows a growth curve with lag phase as the initial phase during which cells adapt to a new environment. Multiple lag phases occur if the media is supplemented with more than one sugar and such type of

growth is referred to as diauxic growth. Following the lag phase is the log phase in which the cell mass and cell number increases exponentially and then the depletion of nutrients starts which indicates the deceleration phase. The accumulation of toxic products results in deceleration phase after which stationary phase commences in which growth rate equals the death rate. The continuous growth kinetics accessed by a perpetual feeding process in which the growth is controlled by the concentration of the rate limiting nutrient [5].

Microbial growth kinetics explains the relationship between the specific growth rate of a microbe and its substrate concentration. Microbial growth kinetics largely depends on the laboratory culture conditions. In batch culture, microbial cell composition and its state change as a function of time and thus the rate of increase in biomass concentration was monitored [6]. Alternatively, in continuous culture the concentration of substrate is at equilibrium and the culture grows at stable physiological state which provides more precise and reproducible data [7, 8]. However, the constant growth conditions represent an artificial growth environment which does not explain many microbial kinetic phenomena. Thus, growth of microbial cells was performed under mixed substrates rather than single substrate to understand the growth kinetics of microorganisms in their natural environment [9].

The substrate such as nutrients (carbon and nitrogen sources), hormones and growth factors influence the growth pattern of microbial and mammalian cells. Substrate limited and substrate-sufficient growth would be observed on the basis of the relative availability of the substrate and the organisms utilize more substrate and energy under substrate sufficient conditions which in turn produces different patterns of product formation. A term describing the residual substrate concentration in Leudeking-Piret model was thus extended in the product formation kinetics [10].

Product of interest is traditionally achieved in the fermentation industry by metabolic engineering of few microorganisms which involves many genetic engineering techniques. The complexity of such genetic modifications and microbial metabolism due to various interconnected pathways urges the need to focus on developing mathematical models for identifying targets of metabolic engineering [11, 12].

Mathematical models are kinetic models which explain the relationship between rates and the concentration of reactants/products and allows to predict the rate of conversion of reactions in to products. This simulated model thus paved way for the optimal design of the operating conditions and operating design of the process for optimal product formation. Qualitative models were mostly used by researchers rather than quantitative models for gene expression systems as quantitative prediction of process parameters are complicated. However, with the advancements in experimental techniques of life sciences and using powerful computer technology, complex mathematical models were developed which is used for the design of various bioprocesses [13]. Industrial Biotechnology largely makes use of such mathematical models and saves time and resources with a clear understanding of strategies to optimize the product yield. Other potential uses of mathematical models include increasing the range of substrates, reduction of undesirable product formation and on the whole optimization of fermentation processes [14, 15].

Studying growth of a microorganism is the basis of biotechnological exploitation of microflora for production of desired product. Optimization of growth of microorganism in a particular media is desirable due to economical and availability of particular growth constituent in a region. Despite this, some microorganisms have specific requirement and they grow in a particular growth media.

The presented overview thus provides a knowledge on the fundamental basics of microbial growth kinetics and energetics which forms the basis for bio-engineering in optimizing, producing and purification of commercially novel products.

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2. Growth kinetics

Classified based on the relationship between product synthesis and energy generation in the cell:

- Growth associated
- Non-growth associated
- Mixed-growth associated

2.1 Growth associated

Growth linked products are formed by growing cells and hence primary metabolites. **Figure 1** clearly shows that product is formed simultaneously with growth of cells. That is product concentration increases with cell concentration. The formation of growth associated product may be described by Eq. (1);

$$\frac{dP}{dt} = r_p = q_p X \tag{1}$$

where P = concentration of product
q_p = specific rate of product formation
X = biomass concentration.

2.2 Non-growth associated

They are formed by cells which are not metabolically active and hence are called secondary metabolites. **Figure 2** clearly shows that product formation is unrelated

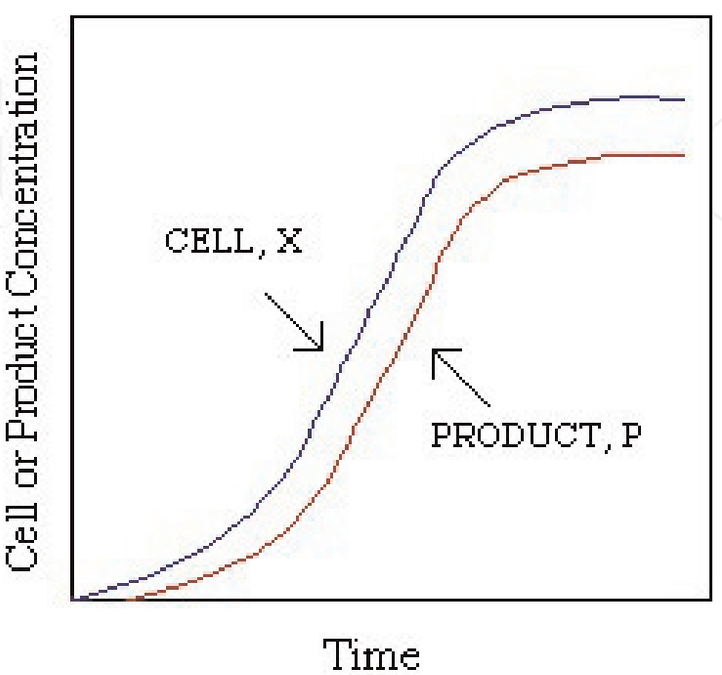

Figure 1.
Growth associated.

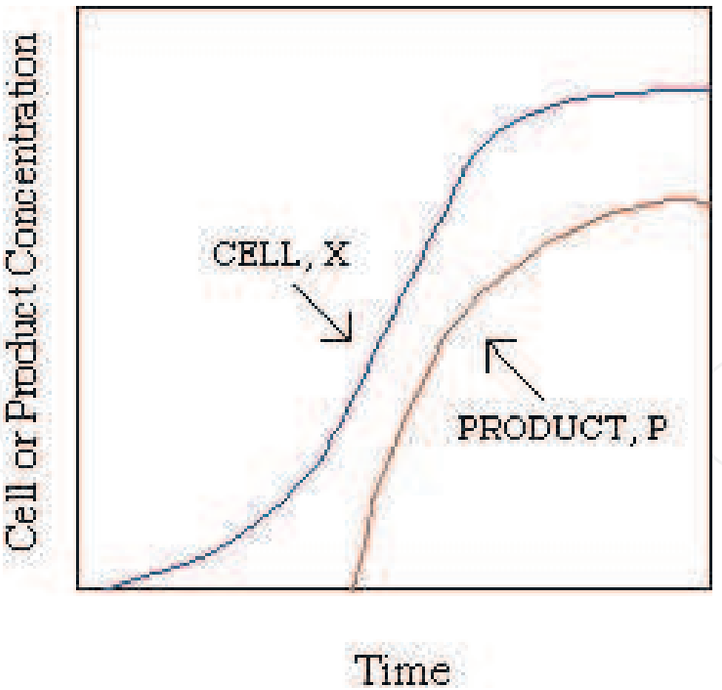

Figure 2.
Non-growth associated.

to growth rate but is a function of cell concentration. The formation of Non-growth associated product may be described by Eq. (2);

$$q_p = \beta = constant \tag{2}$$

2.3 Mixed-growth associated

The product formation from the microorganism depends on both growth and Non-growth associated. It takes place during growth and stationary phases. In **Figure 3**, product formation is a combination of growth rate and cell concentration. The formation of Mixed-growth associated product may be described by Eq. (3);

$$q_p = \alpha_\mu + \beta \tag{3}$$

2.4 Production kinetics

Microbial growth kinetics, i.e., the relationship between the specific growth rate (μ) of a microbial population and the substrate concentration (s), is an indispensable tool in all fields of microbiology, be it physiology, genetics, ecology, or biotechnology, and therefore it is an important part of the basic teaching of microbiology [16]. Unfortunately, the principles and definitions of growth kinetics are frequently presented as if they were firmly established in the 1940s and during the following "golden age" in the 1950s and 1960s the key publications are those of Monod. Monod, logistic, modified logistic model, and Leudeking-Piret models were used to describe the batch growth kinetics of cell. The Monod kinetic model is given as Eq. (4):

$$\mu = \frac{\mu_{max} S}{K_s + \mathrm{S}} \tag{4}$$

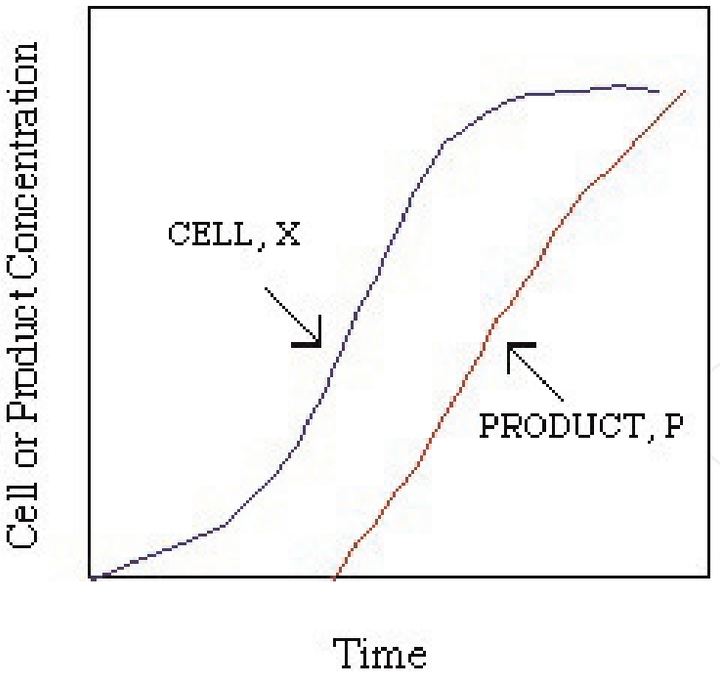

Figure 3.
Mixed growth associated.

where μ is the specific growth rate (h-1), S is substrate concentration (g/L) and K_S and μ_{max} are the Monod constant (g/L) and maximum specific growth rate, (h-1) respectively.

At the end of the lag phase, the growth of microorganisms is well acclimatized for its contemporary environment. Then the cells were multiplied hastily. The major active part of the cell growth curve which is called as the exponential (log) phase is used for the adjudication of kinetic parameters. The period of balanced growth that is the log phase, in which all components of a cell grow at the equivalent rate. Malthus model was also used for the cell growth behavior.

In Contois model, Michaelis constant is directly proportional to cell concentration and specific growth rate is inversely proportional to cell concentration which is described by Eq. (5). The Monod equation was also modified with the maintenance term which was incorporated in the Herbert model (Eq. (6)).

$$\mu = \frac{\mu_{max} S}{K_s \mathrm{X} + \mathrm{S}} \tag{5}$$

$$\mu = (\mu_{max} + m)\left(\frac{S}{K_s + \mathrm{S}}\right) - m \tag{6}$$

where X is cell mass concentration (g/L) and t is time (h). Separation of variables and integrating Eq. (4) yields Eq. (5). The above equations were used to enumerate the cell growth and product accumulation during the batch experiments [17]. The relationship between cell growth and product formation were identified by Leudeking-Piret kinetics.

Leudeking-Piret model (Eq. (7)) was used for kinetic analysis of cell production.

$$\frac{dp}{dt} = \alpha \frac{dx}{dt} + \beta x \tag{7}$$

where α and β are the associated and non-associated growth factor respectively. x and p show the concentration of dry cell weight (DCW) and product

concentration. The Logistic equation was used to analyze the exponential growth phase kinetics while Malthus kinetics was used to express the death phase kinetics (Eqs. (8) and (9)) [16, 18].

$$\frac{dx}{dt} = \mu_m \left(1 - \frac{x}{x_m}\right) x \tag{8}$$

$$\frac{dx}{dt} = \mu \cdot x \tag{9}$$

$$x(t) = \frac{x_0\, exp\,(\mu_m \cdot t)}{\left[1 - \left(\frac{x_0}{x_m}\right)(1 - exp\,(\mu_m \cdot t))\right]} \tag{10}$$

$$1n\left(\frac{x}{x_0}\right)\mu \cdot t \tag{11}$$

where x_m, x_0 and μ_m are the initial DCW or biomass concentration, maximum biomass concentration and maximum specific growth rate of the microorganism, respectively. Also, tm is the required time (seed age) for maximum product concentration by the microorganism. According to Eq. (10), in order to estimate the value of the μm, a plot of $1n\frac{x}{x_m - x}$ against t will yield a straight line that the value of its the slope corresponds to μm and the intercept equals to $1n\left(\frac{x_m}{x_0} - 1\right)$. The substrate and product inhibitory effect on cell growth has been presented by Eq. (11), where x is biomass concentration with respect to time and x_0 is the initial biomass concentration.

$$1n\frac{x}{x_m - x} = \mu_m \cdot t - 1n\left(\frac{x_m}{x_0} - 1\right) \tag{12}$$

The growth pattern of micro-organism followed the modified Logistic model. Maximum cell concentration was obtained for sugarcane bagasse incubated for 48 h when compared to glucose as carbon source. The experimental values deviate slightly towards the end of stationary phase because the modified logistic equation used does not distinguish the decrease in cell density that normally occurs at the end of stationary phase [19]. Substituting Eqs. (8) and (10) into Eq. (7) and integrating, will yield Eq. (13).

$$p(t) = p_0 + \alpha x_0 \left\{ \frac{exp\,(\mu_m \cdot t)}{\left[1 - \left(\frac{x_0}{x_m}\right)(1 - exp\,(\mu_m \cdot t))\right]} - 1 \right\} + \beta \frac{x_m}{\mu_m} 1n \left[1 - \left(\frac{x_0}{x_m}\right)(1 - exp\,(\mu_m \cdot t))\right] \tag{13}$$

Eq. (13) can be rewritten as Eq. (14)

$$p(t) = p_0 + \alpha A(t) + \beta B(t) \tag{14}$$

The value of dx/dt is equal to zero and $x = x_m$ in the stationary phase. Using Eqs. (7) and (13), one can obtain:

$$\beta = \frac{\frac{dp}{dt}(st \cdot phase)}{x_m} \tag{15}$$

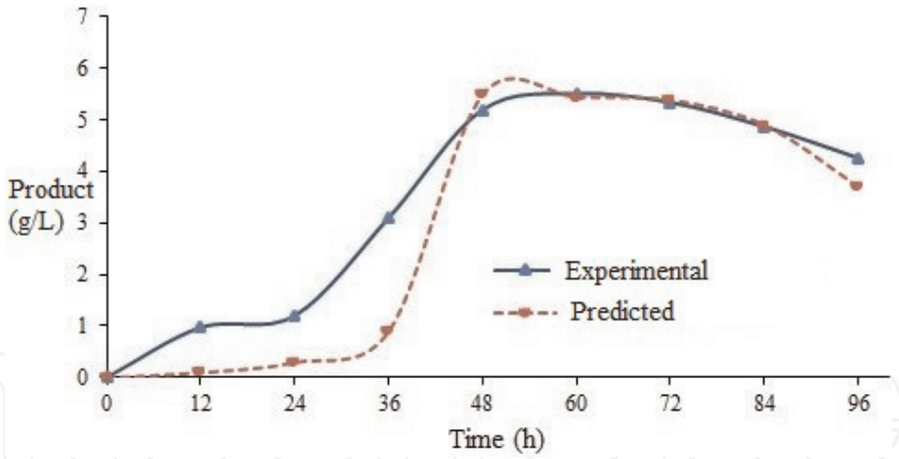

Figure 4.
The kinetic modeling of product production by Leudeking-Piret model using carbon source.

The value of x_m can be obtained from the experimental growth kinetic data and the value of parameter α was obtained from the slope of the linear plot of $p(t) - p_0 - \beta B$ against $A(t)$.

Eqs. (13) and (16) show the kinetic model of product production in the exponential growth phase and death phase, respectively.

$$p(t) = p_0 + \alpha x_0 \, exp\,(\mu \cdot t) + \beta \frac{x_0}{\mu} \, exp\,(\mu \cdot t) \qquad (16)$$
$$= p_0 + \alpha A(t) + \beta B(t)$$

The resulting graph obtained from kinetic modeling of product production by Leudeking-Piret model are shown in **Figure 4**. It is the combination of kinetic models for better agreement between experimental data and model predictions which are employed in cell growth and Product production. The product accumulation mostly adhered to growth-associated kinetic pattern. Matlab ver. 7.12 computer software was used to define the interpretation of growth kinetic parameters.

3. Conclusion

One of the very important practical applications of this model is the evaluation of the product formation kinetics. Mathematical models facilitate data analysis and provide a strategy for solving problems encountered in fermentations. Information on fermentation process kinetics is potentially valuable for the improvement of batch process performance. Finally, the product yields and substrate conversions are criteria with the main attention toward productivity.

Conflict of interest

This is an original work of the authors and it has not been submitted to any other open access publishers previously. Here we have declared that there is no conflict of interest.

Appendices and nomenclature

μ_{max}	maximum specific growth rate (h-1)
X_i	independent variables
X_o	initial biomass concentration (mg/ml)
X_{max}	maximum biomass concentration (mg/ml)
X	biomass concentration (mg/ml)
t	incubation time (h)
DCW	dry cell weight

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